pension *within* the vesicles. This hypothesis agrees with our earlier demonstration that osmium staining blackened the contents of the vesicles (see Figure 2).

Cell fractionation, biochemistry, microphotometry: My associates and I succeeded in obtaining a cell fraction containing the photic vesicles in *Helix aspersa* (Eakin *et al.*, 1974). We were unsuccessful, however, in obtaining publishable biochemical and microphotometric data about the contents of the vesicles.

Ozaki *et al.* (1984, 1986) proved the vesicular pigment to be retinochrome by cell fractionation, biochemistry, fluorescence microscopy, and microphotometry in a brilliant multifaceted investigation of the eyes of a marine gastropod, *Conomulex luhuanus*. Moreover, the authors determined that the photopigment in the microvilli is 11-*cis* rhodopsin. Additionally, they found a difference between the photic vesicles in the perinuclear masses and those more distally situated in the pigmented layer of the eye and perhaps also—my conjecture—in the tips of the photosensory cells. The distal vesicles contain only retinochrome, whereas the aggregated vesicles possess retinochrome *and* aporetinochrome. The investigators speculated that the distal vesicles "act as a direct supplier of retinal to the closely located microvilli, whereas the [perinuclear aggregation of vesicles] serves as a storage place for retinal in retinochrome and for newly formed aporetinochrome."

Similar results in the marine snail *Bulla gouldiana* were reported recently in an abstract of a poster by Bogart *et al.* (1989). Using the fluorescence technique of Ozaki *et al.* (1986) Bogart and her colleagues observed that the "distal segments" of the photoreceptors contained rhodopsin whereas retinochrome was found in the "soma layer" of the receptoral cells. Although photic vesicles were not mentioned, presumably masses of them are situated in the somatic regions of the photosensory cells of *Bulla,* as in other snails.

Bearers of Calcium

Because calcium is an important catalyst in many physiologic processes including photoreception, we investigated the possibility that photic vesicles transport this element in addition to photopigment. Using a non-dispersive X-ray analyzer we showed that the nuclear layer of a *Helix aspersa* eye, wherein lie the masses of vesicles, contains a high concentration of calcium (Eakin & Brandenburger, 1975b). This finding does not, of course, prove that calcium is in the vesicles. Then we (Eakin & Brandenburger, 1980) fixed eyes of *H. aspersa* in glutaraldehyde and treated them with potassium pyroantimonate. Electron microscopy of unstained ultrathin sections of the eyes revealed a dense granule in the center of each photic vesicle. The granules were interpreted as precipitated calcium complexed with pyroantimonate. Credence to this conclusion was provided by the results of experiments in which sections of the same eyes were floated on a solution of the chelating agent EGTA before examining them in an electron microscope. The granules were absent! Conclusion: photic vesicles contain calcium.

A Scenario

In Figure 3 (heretofore unpublished) I summarize the supposed major events in a gastropod eye in which photic vesicles play an important role.

Villar degradation: Light, after passing through the cornea and lens of a snail's eye, strikes the photoreceptoral microvilli. The photoresponse causes the breakdown of the microvillar membranes, especially at the tips (Eakin & Brandenburger, 1982).

Pinocytosis: The debris from the above event is taken up by pinocytosis and phagocytosis by retinal cells, especially type II sensory cells and pigmented supportive cells (Eakin & Brandenburger, 1982; Brandenburger & Eakin, 1983).

Lysosomal digestion: The internalized pinocytic and phagocytic vesicles fuse with primary lysosomes, which contain digestive enzymes (*e.g.,* acid phosphatase), to form secondary lysosomes (Eakin & Brandenburger, 1974; Brandenburger, 1977; Brandenburger & Eakin, 1983).

Synthesis: The products of lysosomal digestion reach the synthetic centers—ER and Golgi apparatus—of type I sensory cells where the recycled molecules become incorporated into aporetinochrome and retinochrome and packaged into photic vesicles.

Photic vesicles: Photic vesicles released from the ER and Golgi cisternae (accelerated by light) are stored in large masses near the nuclei of type I sensory cells. The vesicles are moved distally, supposedly by pulsations of unique cells in the optic capsule that contain smooth muscle fibers (Eakin & Brandenburger, 1972). This process is also accelerated by light.

Smooth endoplasmic reticulum: Upon reaching the distal ends of the sensory cells, the photic vesicles fuse with smooth cisternae beneath the microvilli, releasing retinochrome and other vesicular contents (Eakin & Brandenburger, 1982).

Villar growth: I speculate that the microvilli are regenerated by basal addition of membrane constituents. Molecules of photopigment and perhaps other compounds become incorporated into the microvillar membranes, now ready for light reception again.

ACKNOWLEDGMENTS

I am grateful to the following for advice on this paper: Professors Tomiyuki Hara, Colin Hermans, Ralph Smith, and my research associate Jean Brandenburger. I am especially appreciative of scientific and editorial counsel from my wife, Professor Barbara Nichols Eakin. I acknowledge also the assistance of the Department of Integrative Bi-

ology, especially its artist, Phyllis Thompson Spowart, who prepared Figure 3. Integrative Biology is a successor of the Department of Zoology, my academic home for 60 years (1929–1989).

Added in proof: A. W. Clark (1963, Jour. Cell. Biol. 19:14A) reported many "550 Å spheres" in retinular cells of a snail (*Viviparus maleatus*).

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The Veliger 33(3):215-221 (July 2, 1990)

THE VELIGER

Continuous Reproduction and Episodic Recruitment of *Lacuna vincta* (Montagu, 1803) in the Gulf of Maine

by

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Abstract. Studies of *Lacuna vincta* (Montagu, 1803) limited to the intertidal zone suggest a seasonally defined spawning period. This study, conducted in the subtidal zone, reports continuous spawning year round from Cape Ann, Massachusetts, to Mt. Desert Island, Maine. Larval recruitment occurred in four pulses during an intensive two-year study in Magnolia, Massachusetts. Shell-height frequency distributions of *L. vincta* were bimodal for most months sampled, indicating that overlapping cohorts existed in the population.

INTRODUCTION

Lacuna vincta is a small gastropod with demersal eggs and planktonic larvae that inhabits the rocky littoral and sublittoral zones of the North Atlantic (NORTON, 1971; SMITH, 1973; FRALICK *et al.*, 1974; RUSSELL-HUNTER & MCMAHON, 1975; FRETTER & MANLY, 1977; GRAHAME, 1977; SHATLOCK & CROFT, 1981; SOUTHGATE, 1982; THOMAS & PAGE, 1983; WITMAN, 1985; MANEY, 1986). Adults feed on various species of algae (especially kelps) and lay their eggs in a clear gelatinous ring-shaped capsule on the food plant (SMITH, 1973).

Numerous studies report a single, well-defined spawning period for *Lacuna vincta*, but disagree as to its timing and duration (January–June, SMITH, 1973; January–March, SOUTHGATE, 1982; January–October, RASMUSSEN, 1973; March–June, RUSSELL-HUNTER & MCMAHON, 1975; June–August, THOMAS & PAGE, 1983). SOUTHGATE (1982) suggested that the spawning period may be directly related to latitude. Both SMITH (1973) and SOUTHGATE (1982) derive life-history parameters for populations of *L. vincta* based on the assumption of one cohort per year produced in a single, restricted spawning period.

An intensive long-term study of *Lacuna vincta* in Massachusetts shows that continuous reproduction is occurring in subtidal populations, and observations along the coast of Maine indicate that the habit of continuous spawning extends into higher latitudes. During a two-year period four distinct cohorts existed in Magnolia and overlapped in time. Life-history tables of *L. vincta* based on the assumption of a single cohort derived from a restricted breeding period must be reevaluated in light of the possibility that the underlying data involved overlapping cohorts.

MATERIALS AND METHODS

Forty-eight SCUBA dives were conducted in Popplestone Cove, Magnolia, Massachusetts, from July 1982 to October 1984. During each of these dives, kelp blades were observed for the presence or absence of egg masses. In addition, six randomly selected whole kelp plants (3 each of *Laminaria saccharina* and *L. digitata*) were collected monthly (5 months were missed owing to inclement weather), placed into 0.5-mm mesh nytex bags, and tied off with Velcro straps. This technique sampled all the snails in 0.2 m^2 of the kelp bed population of *Lacuna vincta* because all the snails in the kelp bed were on kelp fronds. In the lab, each sample was fixed in 10% formalin. The snails were counted and separated from the kelp.

Approximately 100 snails from each monthly sample were measured to the nearest 0.1 mm from the tip of the shell apex to the lowest point of the aperture, using an ocular micrometer in a binocular microscope at ×10 magnification. Individuals from each month were placed into shell-height categories of 0.5 mm, and the frequency distributions were plotted as histograms. Using the method of CASSIE (1954) and HARDING (1947) these height measurements were also plotted on probability paper, and the inflection points in the resulting lines were used to distinguish coexisting size classes. For each size class on each sampling date, mean shell height was determined by replotting on probability paper. These mean shell heights were then plotted over time to show more clearly the cohorts of *Lacuna vincta* observed in Popplestone Cove during the 28 months of regular sampling.

During 1985 and 1986, 20 dives were made in Popplestone Cove throughout the year to observe the presence or

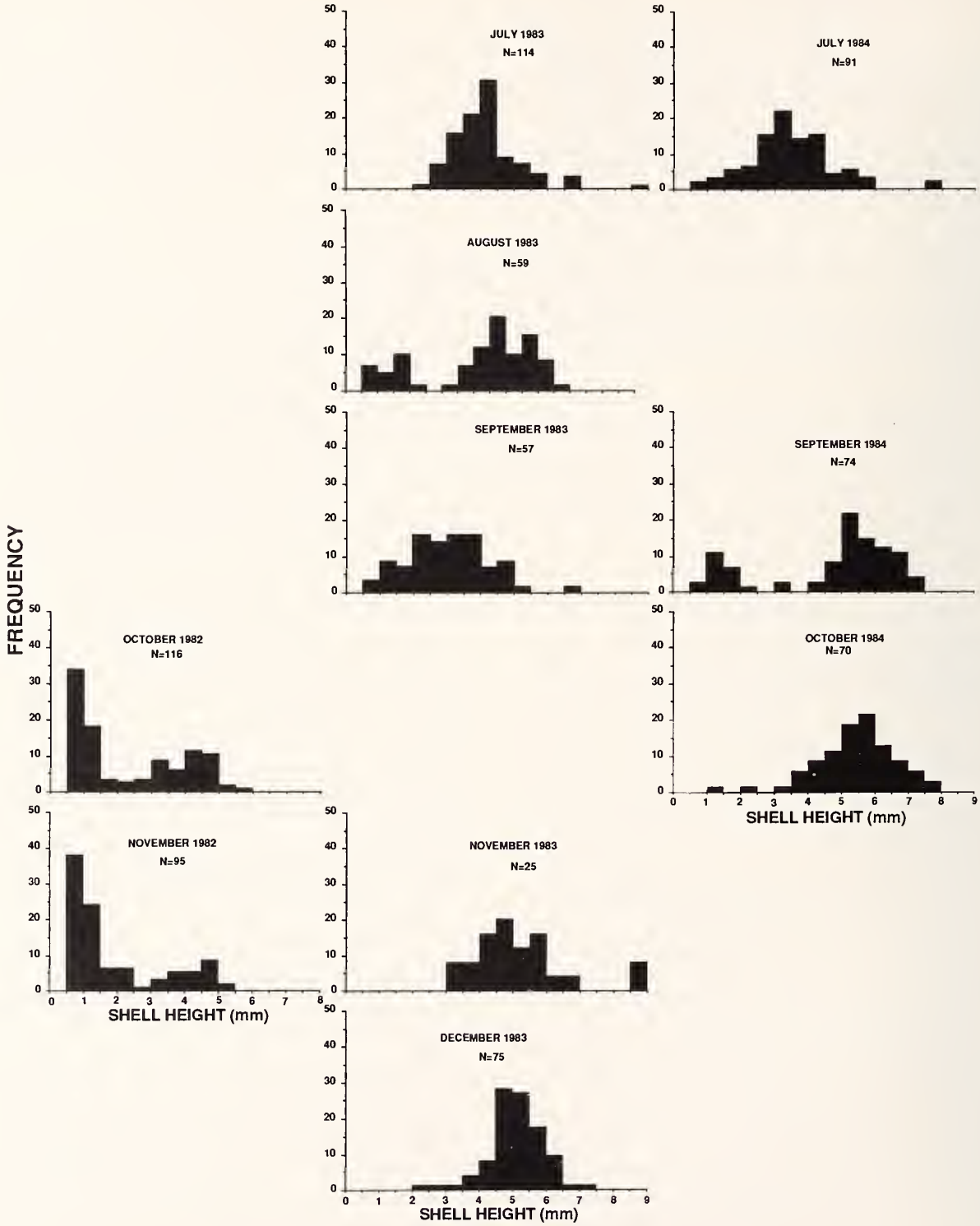
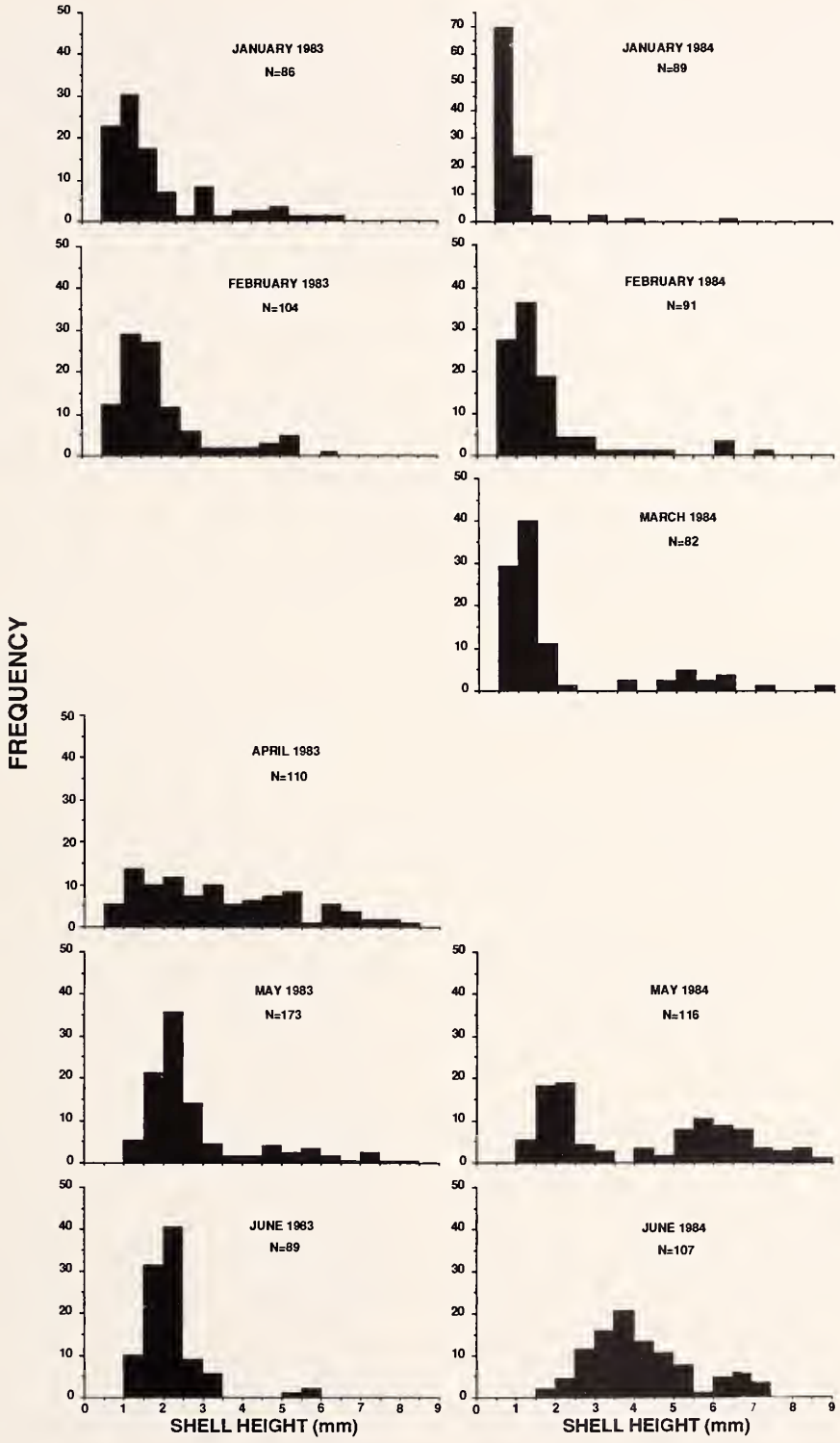


Figure 1

Shell-height frequencies for *Lacuna vincta* in Magnolia, Massachusetts, for October 1982 to October 1984. This figure spans two pages. To follow the temporal sequence, start with October 1982 in the first column, proceed below to November 1982, and then go to January 1983 on the facing page.


JANUARY 1983
N=86
JANUARY 1984
N=89
FEBRUARY 1983
N=104
FEBRUARY 1984
N=91
MARCH 1984
N=82
APRIL 1983
N=110
MAY 1983
N=173
MAY 1984
N=116
JUNE 1983
N=89
JUNE 1984
N=107
FREQUENCY
SHELL HEIGHT (mm)
SHELL HEIGHT (mm)

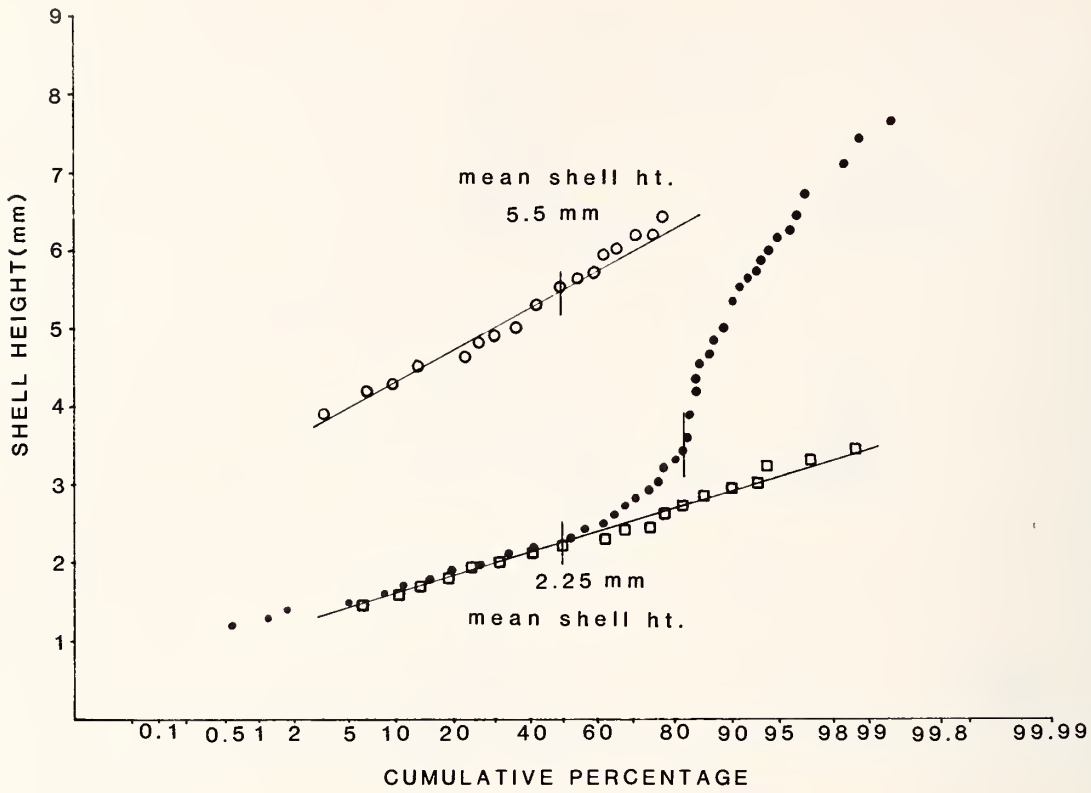


Figure 2

A probability plot of the shell-height data for May 1983. Closed circles depict the entire sample, with a vertical line indicating the inflection point. The two lines drawn with open symbols represent the two data sets on either side of the inflection point, each renormalized to a total of 1.00 to depict two size cohorts.

absence of *Lacuna vincta* egg masses. Trips were made in October, November, and December 1986 to Ogunquit and Mt. Desert Island, Maine, to determine whether *L. vincta* was laying eggs in more northerly latitudes at those times.

RESULTS

We observed *Lacuna vincta* egg masses on blades of the kelps *Laminaria saccharina* and *L. digitata* in Popplestone Cove on all 68 dives throughout the four-year duration of this study (July 1982 to September 1986), indicating that reproduction is continuous at this site.

Shell height of *Lacuna vincta* in Popplestone Cove varied from a minimum of 0.5 mm to a maximum of 13 mm. Frequency distributions of the shell height of *L. vincta* show clear bimodal distributions in some months and possible bimodal distributions in others (Figure 1). To determine whether more than one size class was present at any given time, the cumulative shell-height frequency was plotted on arithmetic probability paper (*e.g.*, Figure 2). A straight line indicates that shell-height frequencies are normally distributed, whereas a sigmoidal curve indicates a bimodal distribution of shell-height frequencies (Harding, 1947; Cassie, 1954). Two normally distributed size classes can be distinguished by considering the portions of the line above and below the inflection points as independent classes. Of 20 samples collected from October 1982 to October 1984, two samples contained a single size class, and 18 samples contained two size classes (Table 1). This suggests that recruitment, unlike reproduction, is *not* continuous for this population. Replotting the portions as lines with 100% cumulative frequency yields for each class the mean shell height, which corresponds to the shell height at the 50% cumulative percentage for the respective replotted lines.

As can be seen by comparing the May 1983 histogram (Figure 1) to the May 1983 probability plot (Figure 2), size classes are more easily distinguished by the inflection points than by inspection of the histograms, but it must be remembered that the probability plot technique is also an "eyeball" method. Misjudgement of an inflection point due to visual error or sampling error may result in the miscalculation of the mean shell height for a size class. It is relatively easy to follow particular size classes over time, especially for *Lacuna vincta* in Popplestone Cove, which typically had two well-separated size classes in each monthly sample.

Five such size classes can be distinguished in a plot of

Table 1

Sample size (n) for monthly samples of *Lacuna vincta* in Popplestone Cove, Magnolia, Massachusetts, and mean shell heights for size classes detected by plots on probability paper. For clarity, mean shell heights are arranged among six arbitrary categories: XSM (0–1.50 mm), SM (1.55–2.00 mm), MED (2.05–3.50 mm), L (3.55–5.00 mm), XL (5.05–5.95 mm), XXL (≥6.00 mm). The solid lines group together size classes that are plotted as presumed cohorts in Figure 3.

Month	n	XSM	SM	MED	L	XL	XXL
Oct-82	116	0.9 (58%)			4.1 (42%)		
Nov-82	95	1.0 (72%)			4.2 (28%)		
Dec-82	—						
Jan-83	86	1.35 (80%)				5.4 (20%)	
Feb-83	104		1.6 (86%)			5.2 (14%)	
Mar-83	—						
Apr-83	110		2.0 (61%)			5.5 (39%)	
May-83	173			2.25 (81%)		5.5 (19%)	
Jun-83	89			2.1 (94%)			6.5 (6%)
Jul-83	114				4.0 (80%)		6.75 (20%)
Aug-83	59	1.3 (24%)			5.0 (76%)		
Sep-83	57			3.0 (98%)			6.9 (2%)
Oct-83	—						
Nov-83	25				4.3 (58%)	5.7 (42%)	
Dec-83	75					5.1 (100%)	
Jan-84	89	0.9 (96%)			4.6 (4%)		
Feb-84	91	1.3 (90%)			4.7 (10%)		
Mar-84	82	1.4 (82%)				5.3 (18%)	
Apr-84	—						
May-84	116			2.1 (50%)			6.2 (50%)
Jun-84	107			3.8 (85%)			6.75 (15%)
Jul-84	91			3.5 (100%)			
Aug-84	—						
Sep-84	74	1.3 (24%)				5.8 (76%)	
Oct-84	70		2.9 (8%)				6.0 (92%)
Total	1823						

mean shell height over time for our samples of *Lacuna vincta* in Popplestone Cove (Figure 3). Two of these size classes appear to be represented in their entirety, and a third, followed over a period of 10 months, showed almost the full range of shell height. The potential for inaccuracies in the determination of monthly mean shell heights precludes the use of this technique to certain quantitative life-history features such as growth curves, but additional analysis may further test the evidence that recruitment in this population is episodic rather than continuous. Linear regression and ANCOVA analysis (Sokol & Rohlf, 1981) were applied to these three size classes to determine whether they should be considered as real cohorts (in this case, sets of individuals that recruited at the same time), which would have distinct origins in time but similar average growth rates. The three regression equations are provided below.

Class A: Shell Height = 0.3801(Time) − 0.1084
Class B: Shell Height = 0.4527(Time) − 2.6601
Class C: Shell Height = 0.5856(Time) − 8.9195

ANCOVA confirms the impression gained from inspection of these equations and Figure 3: the three size classes have distinctively different origins in time as shown by the significant differences in y-intercepts ($P << 0.01$); but they are indistinguishable with respect to average growth rate, since the slopes of the three lines are not significantly different ($P = 0.33$).

To determine whether egg laying was occurring year round at more northern latitudes, trips were made to Maine in October, November, and December 1986. On 29 October 1986, *Lacuna vincta* eggs were observed on the blades of the kelp *Laminaria digitata* that had washed up on Little Hunters Beach in Acadia National Park. *Lacuna vincta*